Acta Biochimica Polonica

Vol. 48 No. 3/2001

623–635

QUARTERLY

Review

Odyssey of *Agrobacterium* T-DNA✪

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Received: 17 May, 2001; accepted: 7 June, 2001

Key words: *Agrobacterium*, T-DNA, DNA transfer, virulence

***Agrobacterium tumefaciens*, a plant pathogen, is characterized by the unique feature of interkingdom DNA transfer. This soil bacterium is able to transfer a fragment of its DNA, called T-DNA (transferred DNA), to the plant cell where T-DNA is integrated into the plant genome leading to "genetic colonization" of the host. The fate of T-DNA, its processing, transfer and integration, resembles the journey of Odysseus, although our hero returns from its long trip in a slightly modified form.**

The soil bacterium, *Agrobacterium tumefaciens*, is a plant pathogen responsible for tumor induction on dicotyledonous plants due to its ability to transfer DNA to the plant cell (reviewed in: de la Cruz & Lanka, 1998; Gelvin, 2000; Hansen & Chilton, 1999; Lartey & Citovsky, 1997; Rossi *et al.*, 1998; Zupan & Zambryski, 1997). In biotechnology this ability is widely used for plant transformation. During tumor induction *Agrobacterium* attaches to plant cells and then transfers part of its DNA to some of these cells. The transferred DNA (T-DNA) which resides on a large Ti (tumor inducing) plasmid, is processed within the bacterium and is exported to the plant where it becomes integrated into the plant genome (reviewed in: Sheng & Citovsky, 1996; Tinland & Hohn, 1995; Tinland, 1996). Proteins encoded by the virulence (*vir*) region of the Ti plasmid regulate T-DNA processing and transfer. Phenolic compounds derived from a wounded plant cell wall induce expres-

✪This work was financed from the State Committee for Scientific Research (KBN, Poland) grant No. 6 P04B 014 18 (1017/P04/2000/18).

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Abbreviations: AS, acetosiringone; LB, left border; NLS, nuclear localization signal; RB, right border; T-DNA, transferred DNA; Ti, tumor inducing; vir, virulence region.

sion of the *vir* region genes. Virulence proteins recognize 25 bp imperfect direct repeats (border sequences) that define the T-DNA. In the presence of VirD1 protein, VirD2 cleaves the border sequence in a site- and strand-specific manner and subsequently becomes covalently attached to the 5′ end of the nicked DNA. The nicked DNA is then displaced from the plasmid producing single-stranded T-DNA. The T-DNA–VirD2 complex and the VirE2 protein are believed to be transferred to the plant through a pilus-like structure containing VirB and VirD4 proteins. In the plant cell, T-DNA becomes coated with the single-stranded DNA-binding protein, VirE2. The T-DNA-protein complex is imported into the nucleus where the T-DNA is integrated into the nuclear genome. Expression of genes located on T-DNA leads to the formation of proteins involved in the production of auxins and cytokinins. These plant hormones cause the tumorous phenotype that is characterized by the ability of the plant cells to proliferate limitlessly and autonomously even in the absence of added phytohormones. Crown gall tumors are characterized by the production of opines (amino-acid derivatives). The biosynthesis of opines is catalyzed by opine synthases, which are encoded by the T-DNA. Opines formed in the tumors can be metabolized by the tumorigenic agrobacteria, but not by most of the other soil organisms. Thus, *Agrobacterium* creates for itself a favorable niche by genetic modification of plant cells, a process called "genetic colonization". All stages of this colonization, including chemotaxis, attachment, induction of virulence region, processing of T-DNA, T-DNA transfer, T-DNA integration, expression of T-DNA genes and changes in the plant phenotype, will be discussed in the following chapters. This will be an odyssey of T-DNA that leaves the *Agrobacterium* cell in the form of nucleic acid and returns from its journey in the form of opines, derivatives of amino acids (Fig. 1).

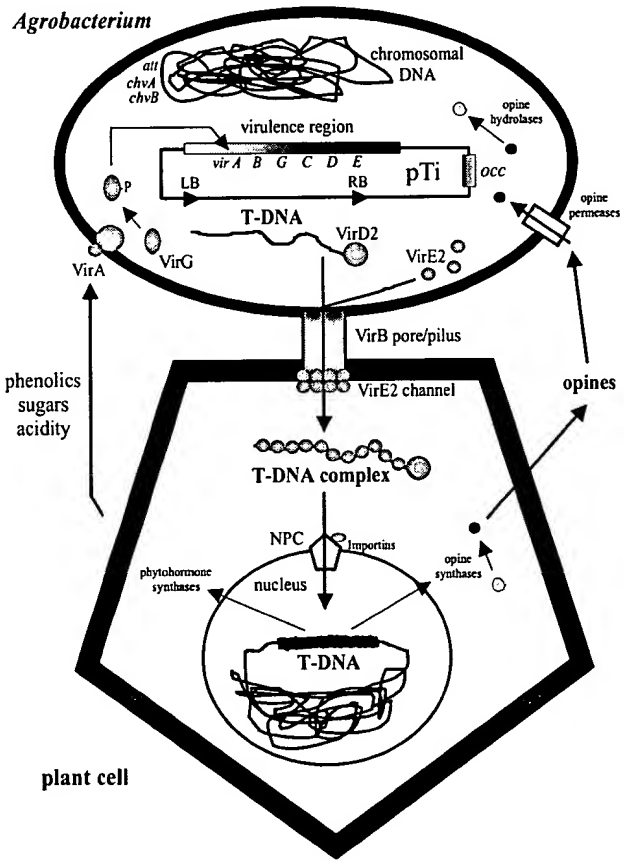

Figure 1. Simpified representation of T-DNA journey from *Agrobacterium* to the plant cell.

LB and RB, left and right border repeats, respectively; NPC, nuclear pore complex. See text for the details.

PART I - PREPARATION FOR JOURNEY

Chemotaxis

Some phenolic compounds act as chemotactic agents that attract *Agrobacterium* cells to the wound site in a plant (Ashby *et al.*, 1988; Parke *et al.*, 1987). Experiments with different *Agrobacterium* strains showed that a highly motile strain exhibited marked, pTi-dependent chemotaxis toward phenolic compounds that strongly induce the *vir* genes, for example acetosyringone, whereas a poorly motile strain did not move toward acetosyringone. This pTi-dependent chemotaxis requires virulence genes, *virA* and *virG* (Shaw *et al.*, 1988). Sugars and amino acids also attract *Agrobacterium* (Hawes *et al.*, 1988).

Attachment

An early step in the process of tumor induction is the attachment of agrobacteria to the plant cells at the wound site. This step occurs prior to or concomitantly with induction of virulence region (see below). Genes involved in the attachment are located in the *Agrobacterium* chromosome, and include *chvA*, *chvB*, *pscA* (or *exoC*) and *att*. Mutations in these loci lead to a loss of virulence towards many plant species.

A group of non-attaching mutants, *att*$^-$, includes both mutants blocked in steps prior to adhesion and mutants which lack the bacterial adhesin (Reuhs *et al.*, 1997). Some of them, AttA1-H deletion mutants, may be defective in sensing the plant signal (see above) or in responding to it. Interestingly, the *attA1-H* genes show homology to the ATP-binding cassette (ABC) present in transport system genes (Matthysse *et al.*, 1996). AttJ$^-$ and AttR$^-$ mutants may lack the bacterial binding site (Matthysse *et al.*, 1996; Reuhs *et al.*, 1997), most likely an acetylated capsular polysaccharide present on the bacterial surface. The *attR* gene shows homology to transacetylases (Reuhs *et al.*, 1997).

Mutations in *chvA* and *chvB* genes cause non-specific alterations in the bacterial surface and result in lack of β1,2-glucans in the bacterial membrane, increased production of extracellular polysaccharides, reduced motility and failure to bind to plant cells. The *chvA* gene codes for an inner membrane protein important for transport of β1,2-glucans to the periplasm (Cangelosi *et al.*, 1989) while the *chvB* gene codes for an inner membrane protein that is involved in β1,2-glucan synthesis (Zorreguieta *et al.*, 1988). Neither the *chvA*$^-$ nor *chvB*$^-$ mutant does produce active rhicadhesin, the 14-kDa Ca^{2+}-dependent protein that mediates the first step in attachment (Swart *et al.*, 1993). The *exoC*$^-$ mutants lack glucose phosphate isomerase and are therefore defective in the production of extracellular polysaccharides (Uttaro *et al.*, 1990). Mutants in the *Agrobacterium* chromosomal *cel* genes (*celABCDE*) are defective in cellulose synthesis and aggregate formation (Matthysse, 1983), thus these mutants bind but loosely to plant wound sites.

In addition to bacterial adhesins and other adhesion factors, several plant factors (plant adhesins) are essential for attachment of agrobacteria to plant cells. These include pectin acceptors, and vitronectin-like and germin-like proteins (Matthysse & Kijne, 1998).

Virulence region induction

Virulence functions are transcriptionally regulated by a two-component gene-regulatory system belonging to a large family of bacterial chemosensors that respond to the chemical environment (Das, 1994). Optimal *vir* gene induction occurs at acidic pH and in the presence of phenolic inducers, such as acetosyringone (AS), that are released by wounded plant cells (Stachel *et al.*, 1985). The *vir* gene regulatory system operates through two monocistronic virulence genes: *virA* and *virG* (Hooykaas & Schilperoort, 1992). The

constitutively expressed *virA* gene produces a protein located in the inner membrane, that responds to plant wound metabolites. VirA is a membrane-spanning protein with an N-terminal periplasmic "sensor" domain (senses AS and related phenolics), a "linker" domain (responds to pH and interacts with ChvE, a sugar-binding protein), a "kinase" domain and a "receiver" domain. Because of its complex structure, VirA can respond to subtle changes in the environment. At suboptimal AS concentrations, VirA can be further stimulated by sugars, crown gall opines or amino acids. The resulting autophosphorylation of VirA protein leads to activation of the intracellular VirG, which is phosphorylated at aspartic acid residue 52 by autophosphorylated VirA (Jin *et al.*, 1990) and becomes the transcriptional activator for all *vir* genes including its own. Promoters of *vir* genes possess one or more 12 bp long "*vir* box" sequences (Winans *et al.*, 1987). Phosphorylated VirG protein binds to "*vir* boxes" and activates transcription of *virBCDEFGH* genes. With the exception of *virH* gene, all other *vir* genes are essential for the *Agrobacterium* virulence, since they are involved in T-DNA processing, transport and integration.

PART II – JOURNEY

Processing of T-DNA

After induction of their *vir* genes *Agrobacterium* cells generate a linear single-stranded DNA, called T-DNA or T-strand, that is the bottom (coding) strand of the T-DNA region of the Ti plasmid (Stachel *et al.*, 1986). This region is flanked by two 25 bp long imperfect direct repeats, termed border sequences. T-DNA borders are recognized and cleaved by two proteins from the *virD* operon: VirD1 and VirD2 (Yanofsky *et al.*, 1986, Stachel *et al.*, 1986; Filichkin & Gelvin, 1993). VirD2 is a cleaving-joining enzyme that cuts the lower strand of the border sequences in a site-specific manner (Yanofsky *et al.*, 1986) at the position between the 3rd and 4th base pair of the 25 bp left and right border repeats (LB and RB). Upon cleavage of the right border, VirD2 becomes covalently attached to the 5′ end of the T-DNA *via* tyrosine residue 29 (phosphothyrosine bond). After this cleavage, the excised T-DNA strand is removed, and the resulting single-stranded gap is repaired, most likely by replacement DNA strand synthesis. The replacement reaction presumably removes the VirD2 molecule attached to the 5′ end of the left border, restoring the circular DNA molecule of the Ti plasmid.

Purified VirD2 protein cleaves single-stranded oligonucleotides containing border sequences (Pansegrau *et al.*, 1993). However, for cleavage of the same sequence in double-stranded plasmid DNA VirD1 protein is required in addition (Scheiffele *et al.* 1995). Initially, VirD1 protein was suggested to function as topoisomerase I (Ghai & Das, 1989), but more recent studies do not support this hypothesis (Scheiffele *et al.*, 1995). Two additional *vir* gene products were also suggested to be involved in T-DNA processing: VirC1 and VirC2. VirC1 has been shown to bind to the "overdrive" site, which lies adjacent to the right border (Toro *et al.*, 1989), and thus to enhance T-DNA border nicking. Other authors suppose that VirC1 and VirC2 are not required for T-DNA processing but rather for efficient T-DNA transfer into most host plants, suggesting that they play a role in T-DNA export (Zhu *et al.*, 2000).

T-DNA transfer

The T-DNA transfer apparatus is encoded by the *virB* operon, which contains 11 genes (Christie, 1997). Each of them, except *virB1*, is essential for tumorigenesis (Berger & Christie, 1994). All 10 essential proteins have been localized to the inner or outer membrane, and most appear either to be integral membrane proteins or to be exported from the cytoplasm. Two VirB proteins, VirB4 and VirB11, are pe-

ripherally bound to the others and located primarily in the cytoplasm, although a small part of VirB4 may span the inner membrane. These proteins show ATPase activity and are thought to provide the energy required for export of other protein subunits, for T-DNA transport, or both. VirB proteins were thought to form pore for T-DNA export. Recently, VirB proteins have been shown to form pili that resemble conjugative pili (Fullner *et al.*, 1996), VirB2 being the major subunit of these pili. VirB7 may help to anchor pilus to the bacterial cell, as it is an outer membrane lipoprotein that forms disulfide bonds with the periplasmically localized VirB9. The VirB mating bridge is probably coupled to the T-DNA complex by the VirD4 protein, which is located in the inner membrane and is absolutely required for transfer. The VirB/VirD4 apparatus delivers the T-DNA–VirD2 complex and VirE2 protein to the plant cell cytoplasm. Once they are within the plant cell, a final T-DNA complex is formed by coating T-DNA with the single-stranded DNA binding protein VirE2. VirE2 binds to T-DNA cooperatively and protects it against nucleolytic degradation by plant nucleases (Rossi *et al.*, 1996). In addition, VirE2 has been shown recently to function as a transmembrane DNA transporter, which suggests that it may form a channel in the plant cellular membrane for transport of the T-DNA–VirD2 complex into the plant cell (Dumas *et al.*, 2001). In the plant cell cytoplasm additional steps are required to transport T-DNA to the plant cell nucleus and to integrate it into the host genome.

Nuclear import of T-DNA

The T-DNA complex in the plant cell consists of single-stranded T-DNA and the bacterial proteins: VirD2 and VirE2, and forms a telephone cord-like helical structure as revealed by electron microscopy analysis (Citovsky *et al.*, 1997). Since the T-DNA presumably does not itself carry targeting signals (Sheng & Citovsky, 1996), its nuclear import is most likely mediated by the agrobacterial proteins accompanying it to the plant cell: VirD2 and VirE2. In eukaryotic cells, active import of proteins and nucleoprotein complexes requires a specific nuclear localization signals (NLS) that are recognized by nuclear import cytosolic factors such as importins. Both, VirD2 and VirE2 contain NLSs that are functional in importing proteins to the plant cell nuclei, and a plant importin that mediates nuclear import of VirD2 has been identified (Ballas & Citovsky, 1997). For nuclear import of the T-DNA complex, the C-terminal NLS of VirD2 has been shown to be the necessary signal both *in vivo* and *in vitro* (Rossi *et al.*, 1993; Ziemienowicz *et al.*, 2001). The potential involvement of the NLSs of the VirE2 protein is less obvious. The nuclear accumulation of ssDNA microinjected into the stamen hairs of *Tradescantia virginiana* or into *Drosophila* and *Xenopus* embryonic cells was found to depend on binding of VirE2 to the ssDNA (Zupan *et al.*, 1996; Guralnick *et al.*, 1996). On the other hand, nuclear import of ssDNA tested in permeabilized tobacco protoplasts or HeLa cells showed strict requirement for both the VirD2 and VirE2 proteins (Ziemienowicz *et al.*, 1999; 2001). This discrepancy may be due to the employment of different techniques for providing the nucleoprotein complexes to the cell and/or due to the use of the VirE2 proteins originating from different *Agrobacterium* strains, which differ in the sequence of their NLSs. It is likely that the VirE2 function in nuclear import of T-DNA is exerted not by its NLSs but rather by imposing a specific structure onto the otherwise unstructured but charged ssDNA, thereby allowing its translocation through the channel of the nuclear pore.

In addition, *in vitro* studies of nuclear import of the T-DNA complexes showed its dependence on cytosolic import factors such as importin and Ran protein, thus indicating that this process follows the so-called classical, importin-dependent import pathway, extensively described for nuclear import of

NLS-carrying proteins (Nakielny & Dreyfuss, 1999).

T-DNA integration

In the plant cell nucleus T-DNA is integrated into the plant genome by illegitimate recombination, a mechanism that joins two DNA molecules that do not share extensive homology. In higher eukaryotic organisms such as plants, illegitimate recombination is the predominant mechanism of DNA integration (Offringa *et al.*, 1990; Paszkowski *et al.*, 1988). Illegitimate recombination of T-DNA in the plant genome has been described a decade ago (Gheysen *et al.*, 1991; Matsumoto *et al.*, 1990; Mayerhofer *et al.*, 1991), but little is known about factors involved in this process.

Some information on T-DNA integration stems from the sequence analysis of several T-DNA insertions and their respective pre-integration sites (Mayerhofer *et al.*, 1991; Tinland *et al.*, 1995). These findings not only provided new details of the mechanism of T-DNA integration, but also suggested a possible role of VirD2 and involvement of other (plant) factors in this process. Based on these findings a modified model for T-DNA integration has been proposed (Tinland *et al.*, 1995). According to this model, first the 3′ end of T-DNA finds some homologies to the plant DNA and anneals. Both, the displaced plant DNA strand and the 3′ overhang of T-DNA, are digested away by nuclease(s). Then, the nucleotide attached to VirD2 finds "microhomology" in the plant DNA and anneals. This annealing brings the electrophilic phosphotyrosine bond of the 5′ end of T-DNA into the proximity of the nucleophilic 3′-OH end of the digested plant DNA. The ends are ligated, the upper strand of plant DNA is degraded and the plant repair machinery synthesizes the upper strand of the T-DNA leading to the completion of the T-DNA integration.

Very little is known about proteins involved in T-DNA integration into the plant cell genome. The VirD2 protein from *Agrobacterium* was suggested to function in T-DNA integration, as it is covalently attached to the 5′ end of T-DNA, pilots T-DNA to the plant nucleus and probably stays attached to T-DNA up to the integration step. Two hypotheses of possible function of VirD2 in T-DNA integration have been proposed: (i) VirD2 acts as an integrase, and (ii) VirD2 acts as a ligase. Analysis of the amino-acid sequence of VirD2 revealed the presence of an H-R-Y motif typical for bacteriophage λ integrase and other site-specific recombinases. An R to G mutation introduced into the H-R-Y motif of the VirD2 protein resulted in a loss of precision of T-DNA integration without any change in its efficiency (Tinland *et al.*, 1995). The unchanged efficiency argues against a function of VirD2 as an integrase, whereas loss of precision of integration (defined as lack of conservation of the 5′-end nucleotide attached to VirD2 in the integrated T-DNA) suggests the importance of VirD2 for the T-DNA integration process. The second hypothesis was based on results of *in vitro* studies of VirD2-mediated cleavage of the right border sequence. VirD2 was found to be able not only to cleave ssDNA at the border sequence but also to ligate the cleaved ssDNA to the 3′ preformed end of another ssDNA molecule (Pansegrau *et al.*, 1993), suggesting a ligase function of VirD2 in T-DNA integration. However, both cleavage and joining reactions were sequence specific while *in vivo* T-DNA integration shows limited requirements for sequence homology. Nevertheless, this hypothesis was widely accepted and, therefore, required extensive examination. The potential function of VirD2 in ligation of 5′ end of T-DNA to the 3′ end of plant DNA has been analyzed *in vitro* (Ziemienowicz *et al.*, 2000) and VirD2 was found not to be able to perform T-DNA ligation. This result suggested that other factors, most probably from plants, are involved in T-DNA ligation/integration. Indeed, such activity was found in plant extracts from tobacco BY-2 cells and pea axes (Ziemienowicz *et al.*, 2000).

Although VirD2 was shown not to act as a ligase for T-DNA, this does not exclude the potential function of VirD2 in other steps of T-DNA integration, for example by recruiting plant enzymes involved in DNA repair or recombination to the site of the integration and/or by interaction with some structural chromatin proteins. Recently, a novel test for identification of plant factors involved in integration of T-DNA has been introduced. It is based on laborious screening of T-DNA tagged *Arabidopsis thaliana* lines to find mutants that are resistant to *Agrobacterium*-mediated transformation (*rat* mutants). This method allowed identification of the first plant factor involved in T-DNA integration, namely histone H2A (Mysore *et al.*, 2000), thus confirming involvement of structural proteins in this process.

PART III – RETURN (WAY HOME)

Expression and functions of transferred genes

T-DNA encodes several proteins that are expressed in the transformed plant cells leading to great changes in the plant phenotype. T-DNA genes can be expressed in the plant cells since they mimic eukaryotic genes. The nontranscribed regions of each transferred gene possess many of the features of plant genes, including typical eukaryotic TATA and CAAT boxes, transcriptional enhancers, and poly(A) addition sites (Binns & Costantino, 1998).

One group of T-DNA genes directs the production of plant growth hormones that are responsible for the proliferation of the transformed plant cells (Binns & Costantino, 1998). The *iaaM* and *iaaH* products direct conversion of tryptophan *via* indolacetamide to indolacetic acid (auxin). The *ipt* product condensates isopentenyl pyrophosphate and AMP (Binns & Costantino, 1998), and host enzymes are presumed to convert the resulting isopentenyl-AMP into the cytokinin zeatin by removal of the phosphoribosyl group and hydroxylation of one methyl group of the isopentenyl moiety. Two other T-DNA genes are thought to play ancillary roles in tumorigenesis. The gene *5* product directs the synthesis of indole-3-lactate, an antagonistic auxin homologue (Korber *et al.*, 1991), while *tml* (also designated gene *6b*) increases the sensitivity of plant cells to phytohormones by a mechanism that remains to be elucidated (Tinland *et al.*, 1992). The latter gene can provoke formation of tumors in certain host plants in the absence of other oncogenes (Hooykaas *et al.*, 1988).

A second set of transferred genes directs the production of bacterial nutrients, called opines, formed by condensation of an amino acid and a keto acid or a sugar (Dessaux *et al.*, 1998). Transformed cells synthesize and secrete significant quantities of particular opines, and the inducing bacteria typically carry genes (outside the T-DNA region and usually on the virulence plasmid) required to catabolize the same opines synthesized by the induced tumor (Guyon *et al.*, 1980; Petit *et al.*, 1983; Petit & Tempé, 1985). Based on the kind of opines produced in the tumors, agrobacteria are classified as octopine, nopaline, succinamopine, and leucinopine strains. There exist at least twenty different opines, and each strain induces and catabolizes a specific set of opines (Petit & Tempé, 1985). For example, octopine-type Ti plasmids direct their hosts to synthesize at least eight opines. The *ocs* gene encodes octopine synthase, which reductively condenses pyruvate with either arginine, lysine, histidine, or ornithine to produce octopine, lysopine, histopine, or octopinic acid, respectively, all of which can be detected in crown gall tumors (Dessaux *et al.*, 1998). The *mas2′* product is thought to condense glutamine or glutamic acid with glucose (although this has not been experimentally demonstrated), while the *mas1′* gene product reduces these intermediates, forming mannopine and mannopinic acid, respec-

tively. The *ags* product catalyzes the lactonization of mannopine to form agropine. Mannopine and agropine also can spontaneously lactamize to form agropinic acid (Dessaux *et al.*, 1998). Thus, the tumors induced by strains harboring octopine-type Ti plasmids can produce four members of the octopine family and four members of the mannityl opine family.

Uptake and catabolism of opines

Opines produced in tumors induced by the *Agrobacterium* infection are then assimilated by the bacteria due to the function of the genes encoded in the opine catabolism region of the Ti plasmid. As an example, the octopine catabolism region of the octopine type Ti plasmid is described below. The octopine catabolic determinants are clustered in two separately transcribed regulons (Schröder *et al.*, 1990). The first contains the regulatory gene *occR*, a member of the *lysR* gene family, which also regulates the conjugal transfer of the plasmid. The OccR negatively regulates its own transcription (independently of the presence or absence of octopine) and positively regulates the transcription of the catabolic genes clustered in the second operon in the presence of octopine (Von Lintig *et al.*, 1994). These genes determine an octopine transport system (*occQ, occM, occJ/T, occP*) (Zanker *et al.*, 1992). OccJ/T is probably the octopine-binding periplasmic protein while the three others appear to be membrane-associated transport proteins. These *occ* genes show similarity to other genes encoding various components of the basic amino acid transport system (HisQ, HisM, HisJ, ArgT and HisP) (Zanker *et al.*, 1992). Interestingly, transport genes of another opine catabolism region of the octopine-type Ti plasmid, *accABCDE*, encode an ABC-type, ATP-driven, periplasmic binding protein-dependent transport system (Dessaux *et al.*, 1998). Downstream of the genes determining the transport system are two genes required for octopine catabolism, *ooxB* and *ooxA*, which encode the two subunits of the octopine hydrolase that cleaves octopine to arginine (Zanker *et al.*, 1992). Arginine is degraded to ornithine and this latter molecule is converted to proline by ornithine cyclodeaminase (OCDase) encoded by the last gene of this operon, *osd* (Schrindler *et al.*, 1989). The other opine degradative regions are closely related to the octopine catabolic region but are organized differently (Dessaux *et al.*, 1998).

In general, each *Agrobacterium* strain catabolizes only opines synthesized by the tumors it induces. In addition, some opines induce conjugal transfer of self-transmissible Ti plasmids between strains of *Agrobacterium* and thereby confer on other strains the ability to catabolize extant opines. Conceivably, *Agrobacterium* strains can create an environmental niche (crown gall tumor synthesizing particular opines) specifically favorable for their growth (Petit & Tempé, 1985). However, although opines probably stimulate growth of the inducing *Agrobacterium* within a crown gall, they apparently do not play a crucial role in their survival (Bouzar & Moore, 1987).

Recently, Kim & Farrand (1998) have shown that *Agrobacterium* strains can be chemoattracted by opines. Chemotaxis is dependent upon plasmids that encode functions required for the catabolism of opine(s). For example, strains with nopalin/agrocinopine-type Ti plasmids are attracted to nopaline and agrocinopines but not to octopine, while strains with octopine-type Ti plasmids are attracted to octopine but not to nopaline or agrocinopines. Not surprisingly, genetic and molecular analyses have localized determinants (e.g. Mcp-like protein genes) to the opine catabolic loci of the Ti plasmids. Opine-mediated chemotaxis could play several roles. First, it could provide a mechanism whereby opine-catabolizing agrobacteria that wander or are swept away from the vicinity of the tumor, can make their way back to the nutrient-rich environment. Second, it may serve to attract *Agrobacterium* strains with some-

what different Ti plasmids. These may then recombine with the Ti plasmids resident in the tumor inducing strain to produce new Ti plasmid types.

CLOSING REMARKS

The ability of *Agrobacterium* to transfer a fragment of its DNA to the plant cell provides a powerful tool for plant biotechnology, and therefore *Agrobacterium*-mediated DNA transfer is one of the most commonly used techniques of plant transformation. In early days this method was restricted only to the dicotyledonous plants, since it was believed that *Agrobacterium* could infect dicots solely. This was due to the use of tumor formation tests as indication of bacterial infection. Later, it came out that although *Agrobacterium* indeed induced tumors only on dicotyledonous plants, it was able to infect monocotyledonous plants as well, but without tumor formation. The host range of *Agrobacterium* is not restricted to plants only. *Agrobacterium* is able to transfer DNA also to other bacterial species from the same family, *Rhizobiaceae*: *Rhizobium* sp., and from another bacterial family, *Enterobacteriaceae*: *Escherichia coli*, as well as to other microorganisms, like yeasts *Saccharomyces cerevisiae* (Bundock & Hooykaas, 1996), filamentous fungi or cultivated mushrooms (de Groot *et al.*, 1998). Most recently, transfer of DNA from *Agrobacterium* to human cells has been documented (Kunik *et al.*, 2001), indicating that although the T-DNA odyssey always starts from and ends at the same point (the *Agrobacterium* cell) it may reach on its way different targets (bacterial, yeast, plant or human cells).

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